Food and Chemical Toxicology 90 (2016) 55–63

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Prediction of the metabolic clearance of benzophenone-2, and its interaction with isoeugenol and coumarin using cryopreserved human hepatocytes in primary culture

Georges de Sousa [a, *], Sophie Teng [b], Romain Salle-Siri [a], Alexandre Pery [b, 1], Roger Rahmani [a, **]

[a] UMR 1331 TOXALIM (Research Centre in Food Toxicology), Institut National de la Recherche Agronomique (INRA), Laboratory of Xenobiotic's Cellular and Molecular Toxicology, 400 route des Chappes, BP 167, 06903 Sophia-Antipolis Cedex, France
[b] INERIS Models for Ecotoxicology and Toxicology Unit, Verneuil-en-Halatte, 60550, France

ARTICLE INFO

Article history:
Received 12 October 2015
Received in revised form
27 December 2015
Accepted 13 January 2016
Available online 29 January 2016

Keywords:
Environmental chemical
Metabolism
Metabolic interaction
Human hepatocytes

ABSTRACT

Benzophenone-2 (BP2) is widely used as a UV screen in both industrial products and cosmetic formulations, where it is frequently found associated with fragrance compounds, such as isoeugenol and coumarin. BP2 is now recognized as an endocrine disruptor, but to date, no information has been reported on its fate in humans. The intrinsic clearance (Cl_{int}) and metabolic interactions of BP2 were explored using cryopreserved human hepatocytes in primary cultures. In vitro kinetic experiments were performed to estimate the Michaelis–Menten parameters. The substrate depletion method demonstrated that isoeugenol was cleared more rapidly than BP2 or coumarin (Cl_{int} = 259, 94.7 and 0.40 µl/min/10^6 cells respectively). This vitro model was also used to study the metabolic interactions between BP2 and isoeugenol and coumarin. Coumarin exerted no effects on either isoeugenol or BP2 metabolism, because of its independent metabolic pathway (CYP2A6). Isoeugenol appeared to be a potent competitive substrate inhibitor of BP2 metabolism, equivalent to the specific UGT1A1 substrate: estradiol. Despite the fact that inhibition of UGT by xenobiotics is not usually considered to be a major concern, the involvement of UGT1A1 in BP2 metabolism may have pharmacokinetic and pharmacological consequences, due to the its polymorphisms in humans and its pure estrogenic effect.

1. Introduction

Benzophenone-2 (BP2) is one of the 12 main benzophenone derivatives (BP1 to BP12), which are widely used as UV screens to protect from light induced damage. These compounds are also used as photo stabilizers in cosmetics and personal care products, as well as sunscreens to protect skin from UV irradiation. Skin creams, lotions and perfumes are complex formulations which include numerous fragrance compounds, nanomaterials and various other elements. As these products are intended for direct application to the skin, human exposure to these complex mixtures can occur through dermal absorption (Janjua et al., 2004). In many EU countries and America, BP2 is no longer permitted for use in sun lotions due to the high concentrations (±10%) traditionally used in these formulations. However, BP2 is still used in plastics, printing ink and cosmetics, to prevent UV induced damage. Often found in wastewater BP-2 is considered an emerging contaminants of concern by the US EPA. It has therefore been suggested that human skin may have daily contact with BP2 (Asimakopoulos et al., 2014) but no information on other possible routes of exposure to BP-2 is available (Buck Louis et al., 2014; Gao et al., 2015). This concept warrants further investigation, not least because the incidence of skin cancer and the photo damaging effects of UV radiation have increased the use of UV protection products. Furthermore, BP2 and other benzophenone derivatives have previously been classed as endocrine disruptors (EDC) (Muncke, 2011; Hass et al., 2012; Kunz

Abbreviations: BP2, benzophenone-2; CHHP, cryopreserved human hepatocytes in primary culture; UDPGA, uridine 5′-diphospho-glucuronic acid; UGT, UDP-glucuronosyltransferase; HPLC, high pressure liquid chromatography; XME, xenobiotic metabolizing enzymes.

* Corresponding author.
** Corresponding author.
E-mail addresses: desousa@sophia.inra.fr (G. de Sousa), rahmani@sophia.inra.fr (R. Rahmani).
[1] Present address: AgroparisTech, Paris, 16 rue Claude Bernard, 75231, France.

http://dx.doi.org/10.1016/j.fct.2016.01.006
0278-6915/

and Fent, 2006). Thirdly presence of BP2 was detected in human tissues samples (Asimakopoulos et al., 2014; Buck Louis et al., 2014; Gao et al., 2015). Several studies have also highlighted the estrogenic effects of BP2 both *in vivo* in rats, *in vitro* in fish models and in coral (Weisbrod et al., 2007; Kim et al., 2011; Downs et al., 2014). Although the potency of this compound as a hormone receptor agonist or antagonist is low, when compared to natural ligands, BP2 has been described as one of the most potent of the BP family, and can elicit harmful biological effects by interacting directly with androgen and estrogen receptors (Molina-Molina et al., 2008), and with the thyroid hormone axis (Schmutzler et al., 2007; Hofmann et al., 2009). A recent study found a clear association of a higher male BP-2 concentration with diminished couple fecundity (Buck Louis et al., 2014). Elimination of these xenobiotics from the body chiefly occurs through Phase I, II and III detoxification processes, however, metabolic interactions between personal care components and BP2 may interfere with its elimination.

Among the multitude of compounds frequently found in cosmetic formulations, coumarin and isoeugenol are commonly associated with BP2. Though coumarin metabolism has been investigated in human and animal models (Felter et al., 2006), BP2 and isoeugenol metabolic pathways are less well documented, and only described in animals.

For all BP congeners, the possible average daily intake (PADI) value was estimated to be 0.33 mg/day/kg but this was possibly an over-estimation (Jeon et al., 2008). For isoeugenol and coumarin, the PADI values were estimated to be 0.2 mg/day/kg and 0.1 mg/day/kg respectively. These were deemed to be far below the values expected to elicit adverse effects in susceptible species. This is particularly true for coumarin, the toxicity of which depends on metabolism via a detoxification pathway in humans (7-hydroxylation) followed by glucurono- and sulfo-conjugation, or an activation process in rat and mouse models (3, 4 epoxidation), which can be inactivated through glutathione conjugation (Lake, 1999).

The pharmacokinetics and metabolism of BP2 and isoeugenol were studied in rats, and it was demonstrated that, after gavage, BP2 was rapidly metabolized to BP2-glucuronide and BP2-sulfate. However, despite the rapidity of the metabolism, estrogenic effects were still observed in the rat uterus (Molina-Molina et al., 2008). Metabolic patterns of BP-2 in numerous cell lines was also investigated showing in HepaRg cells a predominance of a direct glucuronoconjugation. As BP2 is a component of many cosmetics, daily human exposure has been confirmed by biomonitoring, which showed the presence of BP2 in human urine (Asimakopoulos et al., 2014; Buck Louis et al., 2014) Although the metabolic pathways associated with BP2 and isoeugenol have been investigated, little is known regarding their biotransformation in humans or their interactions with other cosmetic components.

The aim of this study was to investigate metabolism of BP2, isoeugenol and coumarin in human hepatocytes and to assess the metabolic interactions induced by isoeugenol and coumarin when co-administered with BP2. The metabolic information obtained in this study may be integrated into physiologically based pharmacokinetic (PBPK) models to predict the fate of BP2 and metabolites in the human body.

2. Materials and methods

2.1. Chemicals

Culture medium and fetal calf serum (FCS) were obtained from Life Technologies Inc. (St Aubin, France). [Ring-14C(U)]-isoeugenol (8 mCi/mmol specific activity), [4-14C]-coumarin (14.5 mCi/mmol specific activity) and [3H]-BP2 (20 Ci/mmol specific activity) were purchased from Bioactif (Strasbourg, France). Radiochemical purity was ±96%. 2,2′,4,4′-tetrahydroxybenzophenone (BP2), isoeugenol and coumarin were from obtained from Sigma–Aldrich Inc. (St Quentin Fallavier, France). All other chemicals were of the highest quality available from commercial sources. Centrifree ultrafiltration tubes (YMT membrane, 30,000 molecular weight cutoff) were supplied by Millipore (Merck, France). All cell culture plastics were obtained from Falcon (Merck Eurolab, Strasbourg, France).

2.2. Human primary hepatocytes preparation

The tissue liver was obtained from the Digestive Unit, Archet 2 Hospital, University Hospital of Nice, France. With informed consent of the tissue donor, and following the ethical guidelines, piece of liver were collected from patient undergoing partial hepatectomy. Patient suffering from infectious disease (hepatitis, HIV) or cirrhotic and steatotic livers were excluded. Only one liver was taken from the program of multi -organ donors due to no transplant receiver. In a studies of Vondran et al. (2008) and Hewes et al. (2006) they indicate that there is no influence of previous chemotherapy on the isolation outcome or subsequent hepatocytes function. Period between chemotherapy and surgery may have allowed functional hepatocyte recovery.

Human hepatocytes were isolated, cryopreserved and thawed as previously described by de Sousa et al. (1997, 1996). Briefly, upon removal of piece of liver (CHU l'Archet, Nice, France), the tissue part tumor free was placed in cold sterile L-15 medium and immediately transferred to the laboratory. Hepatocytes isolation was performed under sterile conditions using a three-step collagenase perfusion. The piece of liver was firstly perfused using an isotonic buffer containing EGTA, followed by the same buffer without EGTA. For tissue dissociation, the liver was perfused with isotonic buffer containing calcium and collagenase 0.05% (Roche Diagnostics, Meylan, France) in recirculation mode for 12–17 min. After a mechanical disruption of the tissue, the cells were washed by centrifugation (35 g, 3 min). The pellet containing mainly hepatocytes is then cryopreserved as described by de Sousa et al.

Human hepatocytes (Table 1) from different donors were cultured into 48-wells Corning® Costar® plates (VWR, France), previously coated with rat tail collagen, at about $\pm 0.6 \times 10^5$ cells/well in an atmosphere of 5% CO_2 and 95% relative humidity in presence of 25–32% of percoll depending the liver to allow only viable to settle down. After 30–60 min, depending the attachment rate of the cells estimated by visual examination, the medium in each well was removed and replaced by 200 μL of Williams E medium containing 10% of fetal bovine serum (FBS), 1 X of a mix of insulin, selenium and transferrin (ITS) and 0.5% of a mix of streptomycin (10,000 μg/mL) and penicillin (10,000 unit/mL) (Life Technologies, Saint-Aubin, France). After 24 h, the cells are washed with William's E medium containing no FCS and no phenol red but supplemented with human serum albumin (HSA, 0.24 μg/ml; which correspond to ±3.6 μM). Incubation with test compounds was performed in the same medium. Previously to the first experiments, BP2, isoeugenol and coumarin stability in the incubation

Table 1
Characteristics of donors. *: Hepatocytes used for interaction studies.

Lot number	Age	Sex	Disease
HuF31*	31	F	Colorectal Metastasis
HuF82	82	F	Colorectal Metastasis
HuM66*	66	M	Colorectal Metastasis
Hu1195	M	49	Not available
Crystal*	No information, the liver was anonymized due to multi-organ removal (French Law).		